

Hosoya *et al.*

Filed: March 29, 2001

Amendment And Reply

Page 9

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version

and  $\gamma$ -glutamyltransferase, and wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is capable of taking up a drug, the cell obtained by treating brain capillary vessels of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells.

### REMARKS

Claims 1-14 are currently pending in the application. Claims 1, 3, 5-7, 9-11 and 13-14 are amended. The amendments find support in the specification and are discussed in the relevant sections below. No new matter is added.

#### The Preliminary Amendment

The Office Action stated that the Preliminary Amendment filed on September 17, 2001 was not entered, because amendments to specification and claims were not made by means of clean, new or replacement paragraphs.

This is not true. The Amendment filed September 17, 2001 clearly provides on page 1 a clean copy of the amendments inserting SEQ ID NOs. The amendments from that paper are resubmitted herein, in new clean form above, and in marked-up copy at the end of this amendment. Entry of the amendments is respectfully requested.

#### Priority

The Office Action states that applicants have not complied with 35 U.S.C. § 119(e) and § 120, by not providing certified copies of foreign applications relied upon for priority. Specifically, the Examiner asks that a copy of the front of Japanese applications 10-296138 and 10-296139, with ribbon and seal, be provided. The Examiner also requests a copy of the PCT Application.

As requested by the Examiner in a telephonic conversation of March 1, 2002, ribbon copies of the two Japanese Applications are filed herewith, as well as a copy of the PCT Application. English translations of Japanese applications 10-296138 and 10-296139 were previously filed on May 29, 2001.

Abstract of the Disclosure

The Examiner objects to the abstract because it contains two paragraphs. A replacement paragraph is provided herein. Entry of the amended Abstract is respectfully requested.

Claim Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 4, 8 and 12 are rejected under 35 U.S.C. § 112, first paragraph, because these claims refer to biological deposits, the Office Action believing that they fail to specify if such deposits were made under the terms Budapest Treaty.

The cells lines FERM BP-6507, FERM BP-6508 and FERM BP-6873 were the subject of a Deposit under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. Applicants note that a copy of the Deposits under the Budapest Treaty for FERM BP-6507 and FERM BP-6508 were provided to the United States Patent and Trademark Office on May 29, 2001, attached to the translations of Japanese patent applications 10-296138 and 10-296139, respectively. Copies of those Deposits, and the Deposit for FERM BP-6873, are provided herewith, along with English translations thereof. Reference to the deposit of FERM BP-6507 occurs in the translation of Japanese application number 10-296138 at page 15, lines 25-28, and page 6, line 27 to page 7, line 3. Reference to the deposit of FERM BP-6508 occurs in the translated copy of Japanese application number 10-296139, at page 14, line 26 to page 15, line 1. Page and line numbers refer to the translations mailed to the United States Patent and Trademark Office on May 29, 2001.

Applicants have also amended the specification, at page 9, lines 4-8, page 10, lines 15-19, page 11, lines 21-26, page 23, lines 5-8 and page 31, lines 5-8, to notify the public that the cell lines were deposited under the terms of the Budapest Treaty. Because copies of the treaty

deposit information for FERM BP-6507 and FERM BP-6508 was provided with the two Japanese applications, and because the deposit information for these two lines and FERM BP-6873 were included with the PCT filing, Applicants submit that the amendments do not represent new matter, and respectfully submit that they be entered and the rejection under 35 U.S.C. § 112, first paragraph, be withdrawn.

Claim Rejections Under 35 U.S.C. § 102

The claims are rejected under 35 U.S.C., in view of various references. Each of these is discussed below.

Claim Rejections under 35 U.S.C. § 102

The claims are rejected under 35 U.S.C. § 102, in view of various references. Each of these is discussed below.

*Claim Rejections In View of Hosoya et al. (2001)*

Claims 1-6 are rejected under 35 U.S.C. § 102(a) as being anticipated by Hosoya *et al.* (*Exp. Eye Res.* 72:163-172, 2001). The Office Action states that the Hosoya *et al.* teaches an immortalized cell “which expresses a temperature sensitive SV40 large T-antigen gene, GLUT-1 transporter and p-glycoprotein derived from the retinal capillary endothelial cells of a transgenic rat into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced.” The Office Action refers to the abstract of the reference in concluding that this reference teaches the TR-iBRB2 cells of the present invention.

Hosoya *et al.* is not prior art, as it was published in 2001, and the instant application claims priority to International Application No. PCT/JP99/05423, filed October 1, 1999 and published on April 13, 2000 as WO 00/20599, and also Japanese Applications 10-296138 (286138/1998) and 10-296139 (286139/1998), both filed on October 2, 1998. Reconsideration and withdrawal of the rejection on the basis of Hosoya *et al.* is therefore respectfully requested.

*Claim Rejections In View of Kitazawa et al.*

Claims 7-10 are rejected under 35 U.S.C. § 102(a) as being anticipated by Kitazawa *et al.* (*Pharm. Res.* 18:16-22, 2001). Specifically, the Office Action states that Kitazawa *et al.* teaches “the immortalized cell which expresses a temperature sensitive SV40 large T-antigen gene, and shows localization of Na<sup>+</sup>-K<sup>+</sup> ATPase and GLUT-1 transporter in the cell membrane and shows localization of the Na<sup>+</sup>-K<sup>+</sup> ATPase in the apical side when cultured in a monolayer” and that this cell is “derived from choroids plexus epithelial cells of a transgenic rat into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 as been introduced” and that this reference teaches the TR-CSFB1 through TR-CSFB5 cells of the present invention.

Kitazawa *et al.* is also not prior art under 35 U.S.C. §102(a), as it was published in 2001, and the present application claims priority to International Application No. PCT/JP99/05423, filed October 1, 1999 and published on April 13, 2000 as WO 00/20599, and also Japanese Applications 10-296138 (286138/1998) and 10-296139 (286139/1998), both filed on October 2, 1998. Reconsideration and withdrawal of the rejection is therefore respectfully requested.

*Claim Rejections In View of Hosoya et al. (2000)*

Claims 11-14 are rejected under 35 U.S.C. § 102(a) as being anticipated by Hosoya *et al.* (*Journal of Drug Targeting* 8:357-370, 2000). The Office Action states that Hosoya *et al.* (2000) teaches “the immortalized cell lines that express GLUT-1 transporter, p-glycoprotein, alkaline phosphatase, and  $\gamma$ -glutamyltransferase”, where the “cell lines were derived from brain capillary endothelial cells of a transgenic rat into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced”, and that this reference therefore teaches the TR-BBB cell lines 1, 5, 6, 11 and 13 of the present invention.

This reference is not prior art under 35 U.S.C. § 102(a), as it was published in 2000, and the present application claims priority to International Application No. PCT/JP99/05423, filed October 1, 1999 and published on April 13, 2000 as WO 00/20599, and also Japanese Applications 10-296138 (286138/1998) and 10-296139 (286139/1998), both filed on October 2,

1998. Applicants therefore request reconsideration and removal of the rejection on the basis of this reference.

*Claim Rejections In View of Hosoya et al. (1999)*

Claims 1-6 are rejected under 35 U.S.C. § 102(b) as being anticipated by Hosoya *et al.* (*Annual Meeting of the Association for Research in Vision and Othomology*, May 9-14, 1999, which appeared as poster 2456-B331 in *Investigative Othomology & Visual Science*, 40(4):S466, Fort Lauderdale, Florida, USA, 1999). The Office Action states that this reference teaches “the immortalized cell which expresses a temperature sensitive SV40 large T-antigen gene, GLUT-1 transporter and p-glycoprotein derived from the retinal capillary endothelial cells of a transgenic rat into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced.” The Office Action concludes that this reference teaches the TR-iBRB2 cells of the invention.

Hosoya *et al.* (1999) is not prior art under 35 U.S.C. § 102(b), as it was published in 1999, and Applicants’ present application claims priority to International Application No. PCT/JP99/05423, filed October 1, 1999 and published on April 13, 2000 as WO 00/20599, and also Japanese Applications 10-296138 (286138/1998) and 10-296139 (286139/1998), both filed on October 2, 1998. Reconsideration and withdrawal of the rejection on the basis of this reference is therefore respectfully requested.

*Claim Rejections In View of Greenwood et al. (1996)*

Claims 1, 3, 6, 11 and 14 are rejected under 35 U.S.C. § 102(b) as being anticipated by Greenwood *et al.* (*J. Neuroimmunol.* 71:51-63, 1996). The Office Action states that this reference discloses “immortalized retinal and brain endothelial cells” which “express the GLUT-1 glucose transporter and the p-glycoprotein . . . and contain an SV40 T-oncogene”, that the “cell lines express all of the markers ubiquitous to endothelia as well as specific markers characteristic of CNS endothelial”, that the “expression of these markers is an inherent property of the CNS

endothelial cells.” The Office Action then concludes that this reference discloses the immortalized cells of claims 1, 3, 6, 11 and 14.

Applicants note however, that this reference does not teach transport of substances such as drugs into the cells, nor that the cells exhibit an inside-outside polarity when cultured *in vitro*. Applicants have amended claims 1, 3, 6-7, 10-11 and 14 to recite these properties, and such amendments are supported in the specification (*e.g.*, inside-outside-polarity is supported at page 1, lines 12-15; page 9, lines 17-19; page 11, lines 2-9; page 12, lines 10-18; page 13, lines 1-9; page 16, line 25 to page 17, line 1; page 33, lines 13-18; page 44, lines 13-17; page 44, line 21 to page 45, line 1; drugs uptake into the cells is supported at page 1, line 26 to page 2, line 3; page 2, lines 7-12, page 2, line 27 to page 3, line 5; page 9, lines 20-27; page 10, lines 3-7, page 11, lines 9-14; page 12, lines 18-23; page 45, lines 1-6; page 45, lines 24 to page 46, line 2). Greenwood *et al.* (1996) does not disclose cells with these properties.

In addition, the cells of Greenwood *et al.* (1996) are not temperature sensitive.. The immortalized cells of the present invention, in contrast, are temperature sensitive, and are immortalized conditionally, that is, their growth is stimulated at 33°C, they are permanently proliferating at 33-37°C, and they terminate proliferation at 39°C due to inactivation of the T-antigen. Applicants have amended claims 1, 5, 9 and 13 to recite this property, and this is supported in the specification at page 5, line 22 to page 6, line 1; page 15, lines 5-8; page 16, lines 2-4 and 22-25; page 17, lines 16-18; page 18, lines 4-6; page 22, lines 14-16 and 21-22; page 22, line 26 to page 23, line 1; page 25, lines 14-17; page 29, lines 6-9; page 30, lines 13-17 and 20-22; page 33 line 27 to page 34, line 3; page 37, lines 10-24; and page 41, lines 3-6. The cells of the present invention can therefore be activated or inactivated depending on the temperature at which they are grown. In contrast, the cells of Greenwood *et al.* (1996) appear to have been cultivated at 37°C, and it is the expression of the large T-antigen only that is temperature sensitive.

Greenwood *et al.* (1996) fails to teach transport of substances such as drugs into the cells, or that the cells exhibit an inside-outside polarity when cultured *in vitro*. This reference also fails to teach that the growth of the cells is temperature sensitive. Greenwood *et al.* (1996)

therefore fails to anticipate each and every limitation of Applicants' claims as is required under 35 U.S.C. § 102. Applicants therefore respectfully request that the rejection of this basis be reconsidered and withdrawn.

Claim Rejections Under 35 U.S.C. § 103

Claims 1-3, 6-7, 10-11 and 14 are rejected under 35 U.S.C. § 103(a) as being unpatentable under Rudland *et al.* (International Application WO97/39117, 1997) and Greenwood *et al.* (U.S. Pat. No. 6,090,624, 2000), further in view of Roux *et al.* (*J. Cell. Physiol.* 159:101-113, 1994) and Villalobous *et al.* (*J. Pharmacol. Exp. Ther.* 282:1109-1116, 1997). The Office Action states that Rudland *et al.* teaches "conditionally immortalized cell lines from brain of transgenic rats expressing the SV40 temperature-sensitive mutant large T-antigen mutant tsA58 gene". The Office Action also states that this reference does not teach "the immortalized cells from the retinal capillary and brain capillary endothelial cells."

Applicants note that the Office Action also states (at page 10, lines 12-13), that Rudland *et al.* does not teach "the immortalized choroid plexus cells with their transgenic rat", thereby effectively stating that Rudland *et al.* does not actually teach any of the cells of Applicants' claims.

In addition, one of the sections of this reference cited in the Office Action (page 32, lines 3-6) teaches cell lines derived from mammary glands, not brain, which were subsequently confirmed to stain for milk fat globule membrane (page 33, lines 9-14). Furthermore, the other section cited by the Office Action (page 39, lines 20-22) teaches primary cultures of brain cells, which were cultured in such a way as to cause cells other than neuronal cells to die out (page 39, last three lines), leaving cells which expressed neuronal markers. This section therefore teaches mammary cells and neuronal cells, not retinal capillary cells, choroid cells or brain capillary endothelial cells.

Regarding the Greenwood *et al.* reference (U.S. Pat. No. 6,090,624), the Office Action states that this reference teaches that "the retinal capillary and brain capillary endothelial cells are considered to have an identical structure (column 1, lines 19-24)", and that the expression of

alkaline phosphatase and gamma-glutamyltransferase are specific to endothelial cells, along with the GLU-1 transporter and p-glycoprotein. Applicants respectfully note that this is not true. The cited section of the Greenwood *et al.* patent actually states that “[I]n the retina, the blood-retina barrier comprises two different types of cells which are anatomically separate. The retinal vascular endothelium, which supplies the anterior portion of the retina, is currently considered to have an identical structure to the cerebral endothelium” (emphasis added). In other words, retinal capillary cells do not have an identical structure to brain capillary cells, rather, the retinal vascular endothelium, which is made up of two different types of cells (one of which is presumeably retinal capillary epithelial cells), is identical in its structure relative to the cerebral endothelium, which is presumeably also made up of two different types of cells (of which one is presumeably the brain capillary endothelium cells). That is, the two tissues are constructed similarly. There is no statement that they are made up of identical types of cells. There is likewise no statement that retinal and brain capillary endothelial cells have the same properties or gene expression.

The Office Action then states that one would be motivated to combine the suggestion of Rudland *et al.* that their invention would be useful in toxicological and pharmacological studies and to make other types of immortalized cells, with the statements of Greenwood *et al.* that their retinal cell lines are capable of conveying a therapeutic substance to the eye and central nervous system.

However, the Examiner has failed to establish a *prima facie* case of obviousness under the requirements of 35 U.S.C. § 103(a). As stated by the Manual of Patent Examining Procedure (MPEP) §§ 2142 and 2143:

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest *all the claim limitations*. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on



applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

(emphasis added). Furthermore, the Court of Customs and Patent Appeals has held that "References relied upon to support a rejection under 35 USC 103 must provide an enabling disclosure, i.e., they must place the claimed invention in the possession of the public." (*In re Payne, Durden, and Weiden*, 203 U.S.P.Q. 245, 255 (C.C.P.A. 1979)).

Applicants' invention is directed to use of immortalized cells as a screen for potential therapeutic agents (see, *e.g.*, page 1, lines 15-23, page 2, lines 7-12 and lines 23-27). Greenwood *et al.* (2000) teaches immortalized cell lines where the cells "integrate in vivo into the retina" (claim 1, col. 18, line 5), and "which are capable of being implanted in the retina and of conveying a substance of therapeutic interest into the eye and central nervous system" (col. 1, lines 8-13). That is, Greenwood *et al.* is directed to cell lines to be used in gene therapy, not as screens. Rudland *et al.* teaches immortalized rat cell lines, and states a general desire to use the cell lines in drug validation (page 11, lines 6-11). That is, Rudland *et al.* teaches several lines of immortalized cells (none like Applicants'), and suggests drug screening as a potential use of such cells. Greenwood *et al.* (2000) teaches production of immortalized cells for gene therapy. Contrary to the statements contained in the Office Action, there is no motivation to combine these two references.

To establish a *prima facie* case of obviousness, the Federal Circuit has stated in *In re Geiger* (815 F.2d 686, 688, 2 U.S.P.Q.2d 1276, 1278 (Fed. Cir. 1987) that "[o]bviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching[,] suggestion or incentive supporting the combination." In *Geiger*, the PTO had failed to establish a *prima facie* case of obviousness, and the court went on to say that "[a]t best, in view of these disclosures, one skilled in the art might find it obvious to try various combinations of these known . . . agents. However, that is not the standard of 35 U.S.C. §103." *Id.*, at 1278.

Furthermore, the Federal Circuit has long held that "obvious to try" does not constitute "obviousness." The court in *In re O'Farrell* (853 F.2d 894, 7 U.S.P.Q.2d 1673 (Fed. Cir. 1988))

made an excellent distinction between these two concepts. Judge Rich noted that “[a]ny invention that would in fact have been obvious under §103 would also have been, in a sense, obvious to try. The question is: when is an invention that was obvious to try nevertheless nonobvious?” (*Id.* at pages 1680-81). He went on to state that

The admonition that ‘obvious to try’ is not the standard under § 103 has been directed mainly at two kinds of error. In some cases, what would have been ‘obvious to try’ would have been to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful. [4 case cites omitted]. In others, what was ‘obvious to try’ was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it.

(*Id.*, at 1681). The prior art cited by the Examiner clearly falls into Judge Rich’s second category. The mere fact that references can be combined does not render the resultant combination obvious unless the prior art also suggest the desirability of the particular combination. *Berghauser v. Dann*, *Comr. Pats.*, 204 U.S.P.Q. 393 (Dist. DC 1979); *ACS Hospital Systems, Inc. v. Montefiore Hospital*, 221 U.S.P.Q. 929 (Fed. Cir. 1984). Citing references which merely indicate that isolated elements and/or features recited in the claims are known is not a sufficient basis for concluding that the combination of claimed elements would have been obvious. *Ex parte Hiyamizu*, 10 U.S.P.Q.2d 1393 (Bd. Pat. App. & Inter. 1988). The statement in *Rudland et al.* of a general desire that the cells disclosed therein could be used as screens cannot render obvious Applicants’ enabled claims to different immortalized cells that have actually been shown to behave as do native cells, even in combination with the gene therapy cells disclosed in *Greenwood et al.* (2000).

Furthermore, the addition of *Roux et al.* and *Villabous et al.* fail to cure this deficiency. These references merely discuss various features of brain capillary endothelial cells and choroid plexus cells, respectively. Neither teaches nor suggests anything regarding the establishment of immortalized versions of such cells for drug screening.

For the reasons described above, the references cited in the Office Action fail to render obvious Applicants' claims, and the rejection on this basis should be reconsidered and withdrawn.

Applicants submit that in view of the foregoing remarks, all issues relevant to patentability raised in the Office Action have been addressed. Applicants respectfully request the withdrawal of rejections over the claims of the present invention.

Respectfully submitted,

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MARKED-UP VERSION OF AMENDMENTS:

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Please replace the paragraph at page 9, lines 1 through 8 with the paragraph below, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

transgenic animals. In particular, the present invention relates to established cells which express a temperature sensitive SV40 large T-antigen, GLUT-1 transport carrier, and p-glycoprotein. Cell deposited in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, the Ministry of International Trade and Industries, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure, under the deposition number FERM BP-6507 can be given as such established cells.

Please replace the paragraph at page 10, lines 8 through 19 with the paragraph below, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

The present invention also relates to established cells derived from choroid plexus epithelial cells of such a transgenic animal. Specifically, the present invention relates to established cells expressing a temperature sensitive SV40 large T-antigen gene, showing localization of Na<sup>+</sup> -K<sup>+</sup> ATPase and GLUT-1 transport carriers in the cell membrane, and when cultured in a monolayer, showing the localization of Na<sup>+</sup> -K<sup>+</sup> ATPase in the apical side. The cells deposited in National Institute of Bioscience and Human-Technology, Agency of

Industrial Science and Technology, the Ministry of International Trade and Industries, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure, under the deposition number FERM BP-6508 can be given as such established cells.

Please replace the paragraph at page 11, lines 15 through 26 with the paragraph below, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

Furthermore, the present invention relates to established cells derived from brain capillary endothelial cells of such a transgenic animal. Specifically, the present invention relates to established cells which express a temperature sensitive SV40 large T-antigen, maintain an alkaline phosphatase activity and  $\gamma$ -glutamyltransferase activity, and express a scavenger receptor, GLUT-1 transporter and p-glycoprotein. The cell line deposited in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, the Ministry of International Trade and Industries, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure, under the deposition number FERM BP-6873 can be given as such an established cell.

Please replace the paragraph at page 20, lines 1 through 27 with the paragraph below, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

by being together in a cage. The ova at pronucleous stage were collected at 32 hours after the hCG administration by oviduct perfusion. A mKRB solution

(Toyoda Y. and Chang M.C., J. Reprod. Fertil., 36, 9-22 (1974)) was used for the oviduct perfusion and incubation of ova. The collected (fertilized) ova were treated by an enzyme in an mKRB solution containing 0.1% hyaluronidase (Hyaluronidase TypeI-S, made by Sigma Co.) at 37°C for 5 minutes to remove cumulus cells. After washing three times with the mKRB solution to remove the enzyme, the fertilized ova were stored in a CO<sub>2</sub> incubator (5% CO<sub>2</sub>-95% air, 37°C, saturated humidity) until DNA microinjection. A DNA solution was microinjected into the male pronucleus of the rat (fertilized) ova thus prepared. 228 ova after microinjection were transplanted in nine recipients (foster mothers) and 80 pups were obtained. The integration of the DNA was analyzed with DNA prepared from tails of the rats immediately after weaning by the PCR method (primers used: tsA58-1A, 5'-TCCTAATGTGCAGTCAGGTG-3', SEQ ID NO:1 (corresponds to 1365-1384 sites), tsA58-1B, 5'-TGACGAGCTTTGGCACTTG-3', SEQ ID NO:2 (corresponds to 1571-1590 sites)). As a result, 20 rats (6 male, 8 female, and 6 unknown sexuality) were identified to have the gene introduced. Among these rats, 11 transgenic rat lines (male lines: #07-2, #07-5, #09-6, #12-3, #19-5, female lines: #09-7, #11-6, #12-5, #12-7, #18-5, #19-8) which survived as long as 12 weeks after elapse of the sexual maturation period were obtained. These G0 generation transgenic rats were mated with Wistar rats and established 2 lines of male founders (#07-2, #07-5) and 3 lines

Please replace the paragraph at page 23, lines 5 through 8 with the paragraph below, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

TR-iBRB2 was deposited in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, the Ministry of International Trade and Industries, under the terms of the Budapest Treaty on the

International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. The deposition number is FERM BP-6507.

Please replace the paragraph at page 31, lines 5 through 8 with the paragraph below, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

TR-CSFB3 was deposited in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, the Ministry of International Trade and Industries, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. The deposit number is FERM BP-6508.

Please replace the paragraph at page 47, lines 3 through 4 with the paragraph below, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

Name and address of the organization in which the microorganisms have been deposited under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure:

Please replace the paragraph at page 47, lines 14 through 15 with the paragraph below, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

Name and address of the organization in which the microorganisms have been deposited under the terms of the Budapest Treaty on the International

Recognition of the Deposit of Microorganisms for the Purpose of Patent

Procedure:

Please replace the paragraph at page 47, lines 25 through 26 with the paragraph below, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

Name and address of the organization in which the microorganisms have been deposited under the terms of the Budapest Treaty on the International

Recognition of the Deposit of Microorganisms for the Purpose of Patent

Procedure:

Please replace the Abstract at page 52, lines 3 through 25 with the Abstract below, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

Established cells derived from retinal capillary endothelial cells, choroid plexus epithelial cells, or brain capillary endothelial cells or a transgenic animal carrying a large T-antigen gene of an SV40 temperature sensitive mutant tsA58 are disclosed. The cell line derived from retinal capillary endothelial cells expresses a temperature sensitive SV40 large T-antigen, GLUT-1 transporter, and p-glycoprotein. The cell line derived from choroid plexus epithelial cells expresses a temperature sensitive SV40 large T-antigen gene and shows localization of Na<sup>+</sup> -K<sup>+</sup> ATPase and GLUT-1 transporter in the cell membrane. When cultured in a monolayer, it shows the localization of Na<sup>+</sup> -K<sup>+</sup> ATPase in the apical side. The cell line derived from brain capillary endothelial cells expresses a temperature sensitive SV40 large T-antigen, GLUT-1 transporter, p-glycoprotein, alkaline phosphatase, and  $\gamma$ -glutamyltransferase. A method of



establishing immortalized cells by subculturing cells obtained from retinal capillary endothelial cells, choroid plexus epithelial cells, or brain capillary endothelial cells of the above-described transgenic animal are disclosed. These cells are useful in screening drugs regarding safety and efficacy thereof, and developing method for diagnosing and treating diseases relating to nutrition metabolism in retinal tissues and brain on cellular level studies.

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

Please amend claims 1, 3, 5-7, 9-11 and 13-14 as follows:

1. (Amended) [An] A conditionally immortalized cell established from a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced, and wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is capable of taking up a drug.
3. (Amended) An established cell derived from retinal capillary endothelial cells, which expresses a temperature sensitive SV40 large T-antigen gene, GLUT-1 transporter, and p-glycoprotein, and wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is capable of taking up a drug.
5. (Amended) A method of establishing a conditionally [an] immortalized cell which expresses a temperature sensitive SV40 large T-antigen gene, GLUT-1 transporter, and p-glycoprotein, the method comprising treating retinal capillary vessels of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells.
6. (Amended) An established cell which expresses a temperature sensitive SV40 large T-antigen gene, GLUT-1 transporter, and p-glycoprotein, and wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is capable of taking up a drug, the cell obtained by treating retinal capillary vessels of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells.

7. (Amended) An established cell derived from choroid plexus epithelial cells, wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is capable of taking up a drug, which expresses a temperature sensitive SV40 large T-antigen gene, shows localization of  $\text{Na}^+\text{-K}^+$  ATPase and GLUT-1 transporter in the cell membrane, and when cultured in a monolayer, shows the localization of  $\text{Na}^+\text{-K}^+$  ATPase in the apical side.
9. (Amended) A method of establishing a conditionally [an] immortalized cell which expresses a temperature sensitive SV40 large T-antigen gene, shows localization of  $\text{Na}^+\text{-K}^+$  ATPase and GLUT-1 transporter in the cell membrane, and when cultured in a monolayer, shows the localization of  $\text{Na}^+\text{-K}^+$  ATPase in the apical side, the method comprising treating choroidal epithelium tissues of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells.
10. (Amended) An established cell which expresses a temperature sensitive SV40 large T-antigen gene, wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is capable of taking up a drug, and shows localization of  $\text{Na}^+\text{-K}^+$  ATPase and GLUT-1 transporter in the cell membrane, and when cultured in a monolayer, shows the localization of  $\text{Na}^+\text{-K}^+$  ATPase in the apical side, which is obtained by treating choroidal epithelium tissues of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells.
11. (Amended) An established cell derived from brain capillary endothelial cells, wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is

capable of taking up a drug, which expresses a temperature sensitive SV40 large T-antigen, GLUT-1 transporter, p-glycoprotein, alkaline phosphatase, and  $\gamma$ -glutamyltransferase.

13. (Amended) A method of establishing a conditionally [an] immortalized cell which expresses a temperature sensitive SV40 large T-antigen gene, GLUT-1 transporter, p-glycoprotein, alkaline phosphatase, and  $\gamma$ -glutamyltransferase, the method comprising treating brain capillary vessels of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells.
14. (Amended) An established cell which expresses a temperature sensitive SV40 large T-antigen gene, GLUT-1 transporter, p-glycoprotein, alkaline phosphatase and  $\gamma$ -glutamyltransferase, and wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is capable of taking up a drug, the cell obtained by treating brain capillary vessels of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells.